Exhibit A

The invention Shows Synergistic Results
(From Tables 1 and 2, *see* Specification, pages 48 and 50)

Property	E217A	W215A	WE
PA/FC *	40.06	170	2865
Fibrinogen k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	0.27	0.034	0.00089
Fibrin k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	0.15	0.053	0.0021
Protein C + TM k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	0.14	0.075	0.033
PAR1 k_{cat}/K_m ($\mu M^{-1}s^{-1}$)	0.66	1	0.026
Antithrombin III k_{on} ($\mu M^{-1}s^{-1}$)[d]	1	0.56	0.0040

*The PA/FC here are calculated from the data shown in Tables 1 and 2. The term "PA/FC ratio" as used herein refers to the ratio of the percent of wild-type protein C activation (PA) activity remaining in a thrombin variant relative to the percent of wild-type fibrinogen clotting (FC) activity remaining in the thrombin variant. A value of PA/FC greater than 1.0 indicates that the thrombin variant has reduced procoagulant fibrinogen cleavage activity relative to the residual anticoagulant activity resulting from protein C activation. *See* Specification 16.

Comparative Data Between Cited References and the Invention

Property	The Primary Reference E229A (E217A)	The Secondary Reference W215A	The Invention WE
PA/FC *	19.1	170	2865

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Proceedings of the National Academy of Sciences, Vol 92, 5977-5981,

ARTICLE

An Allosteric Switch Controls the Procoagulant and Anticoagulant Activities of Thrombin

QD Dang, A Vindigni and ED Cera

Thrombin is an allosteric enzyme existing in two forms, slow and fast, that differ widely in their specificities toward synthetic and natural amide substrates. The two forms are significantly populated in vivo, and the allosteric equilibrium can be affected by the binding of effectors and natural substrates. The fast form is procoagulant because it cleaves fibrinogen with higher specificity; the slow form is anticoagulant because it cleaves protein C with higher specificity. Binding of thrombomodulin inhibits cleavage of fibrinogen by the fast form and promotes cleavage of protein C by the slow form. The allosteric properties of thrombin, which has targeted two distinct conformational states toward its two fundamental and competing roles in hemostasis, are paradigmatic of a molecular strategy that is likely to be exploited by other proteases in the blood coagulation cascade.

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Abstract #2

Article

Biochemistry and Molecular Biology International
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Non-synergistic interactions between strong allosteric effectors and human embryonic and adult haemoglobins

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Abstract:

The binding of two strong allosteric effectors (2,3 Diphosphoglycerate D.P.G., and Bezafibrate, Bzf) to both adult and the three human embryonic haemoglobins, either individually or in combination, have been studied in detail. The adult protein exhibits one binding site for D.P.G and two for Bzf. When both effectors are present simultaneously their effects are simply additive. The same qualitative pattern of binding is observed in the case of the three human embryonic haemoglobins, although with different binding constants. The lack of synergism between these effectors and the different binding affinity expressed by these proteins are discussed in terms of the known amino acid sequence differences.

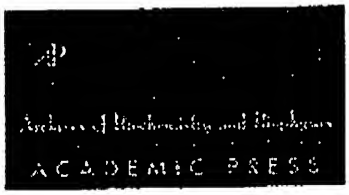
Keywords:

haemoglobin, embryonic, allosteric, effectors

Abstract #3

ingentaconnect

Kinetic Characterization of a T-State ofAscaris suumPhosphofructokinase with Heterotropic Negative Cooperativity by ATP Eliminated



Authors: Jagannatha Rao G.S.[1]; Cook P.F.[2]; Harris B.G.[1]

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Publisher: Academic Press

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Abstract:

The affinity analogue, 2′,3′-dialdehyde ATP has been used to chemically modify the ATP-inhibitory site of**Ascaris suum**phosphofructokinase, thereby locking the enzyme into a less active T-state. This enzyme form has a maximum velocity that is 10% that of the native enzyme in the direction of fructose 6-phosphate (F6P) phosphorylation. The enzyme displays sigmoid saturation for the substrate fructose 6-phosphate ($S_{0.5}$(F6P) = 19 mM andn_H= 2.2) at pH 6.8 and a hyperbolic saturation curve for MgATP with a$K_m$identical to that for the native enzyme. The allosteric effectors, fructose 2,6-bisphosphate and AMP, do not affect the$S_{0.5}$for F6P but produce a slight (1.5- and 2-fold, respectively)V-type activation

with K_a values (effector concentration required for half-maximal activation) of 0.40 and 0.24 mM, respectively. Their activating effects are additive and not synergistic. The kinetic mechanism for the modified enzyme is steady-state-ordered with MgATP as the first substrate and MgADP as the last product to be released from the enzyme surface. The decrease in V and V/K values for the reactants likely results from a decrease in the equilibrium constant for the isomerization of the E:MgATP binary complex, thus favoring an unisomerized form. The V and V/K_{F6P} are pH dependent with similar pK values of about 7 on the acid side and 9.8 on the basic side. The microenvironment of the active site appears to be affected minimally as evidenced by the similarity of the pK values for the groups involved in the binding site for F6P in the modified and native enzymes.

Keywords: Ascaris suum; phosphofructokinase; T-state; R-state; dialdehyde-ATP; V-type activation; allosteric effectors

Language: English

Document Type: Research article

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